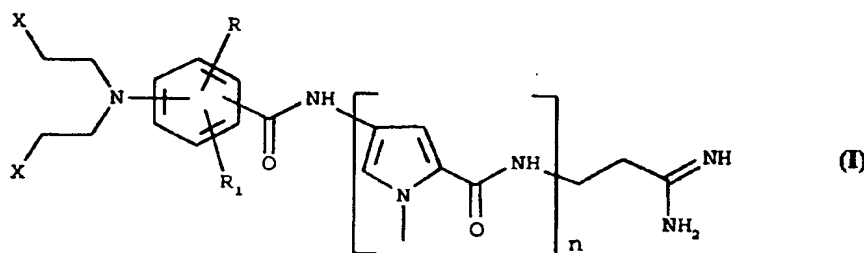




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<p>(21) International Application Number: PCT/EP96/02659</p> <p>(22) International Filing Date: 19 June 1996 (19.06.96)</p> <p>(30) Priority Data: 9514993.6 21 July 1995 (21.07.95) GB</p> <p>(71) Applicant (for all designated States except US): PHARMACIA & UPJOHN S.P.A [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): COZZI, Paolo [IT/IT]; Via Zanella, 48/5, I-20133 Milan (IT). BERIA, Italo [IT/IT]; Via G. Matteotti, 39, I-45030 Villamarzana (IT). CAPOLONGO, Laura [IT/IT]; Piazzale Siena, 18, I-20146 Milan (IT). FRANZETTI, Cristina [IT/IT]; Via De Gasperi, 4, I-21023 Besozzo (IT).</p>	<p>(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published With international search report.</p>	

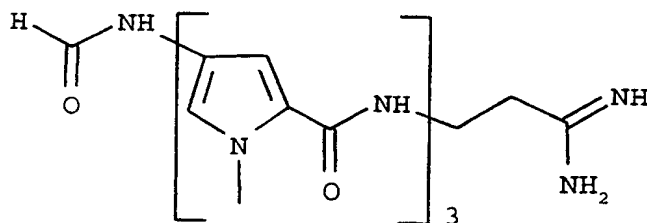
(54) Title: BIS-(2-HALOETHYL)AMINOPHENYL SUBSTITUTED DISTAMYCIN DERIVATIVES AS ANTITUMOR AND ANTIVIRAL AGENTS



(57) Abstract

Novel antitumor and antiviral agents of formula (I) wherein n is 2, 3 or 4; one of R and R₁ is hydrogen, C₁-C₄ alkyl, CF₃ or C₁-C₄ alkoxy and the other is independently CF₃, C₁-C₄ alkyl or C₁-C₄ alkoxy; and X is halogen; and the salts thereof are disclosed.

The present invention refers to novel antitumor alkylating and antiviral agents related to the known antibiotic distamycin A.



which belongs to the family of the pyrroleamidine antibiotics and is reported to interact reversibly and selectively with DNA-AT sequences interfering with both replication and transcription [Nature 203, 1064 (1964); FEBS Letters 7 (1970) 90; Prog. Nucleic Acids Res.Mol.Biol., 15, 285 (1975)].

DE-A-1795539 describes the preparation of distamycin derivatives in which the formyl group of distamycin is replaced by hydrogen or the acid residue of an organic C₁-C₄ aliphatic acid or of cyclopentylpropionic acid.

EP-B-246868 describes distamycin A analogs in which the distamycin formyl group is substituted by aromatic, alicyclic or heterocyclic moieties bearing alkylating groups.

20 It has now been found that a selected class of compounds falling within the general chemical formula of EP-B-246868 has more valuable biological properties than the related prior art compounds.

Accordingly the present invention provides new site specific
25 nitrogen mustards, a process for their preparation,
pharmaceutical compositions containing them and their use in

groups are each other preferably in the meta or para positions.

R and R₁ can be on any of the free carbon atoms of the phenyl ring, not on the same carbon atom of course. Preferably one of R and R₁ is hydrogen or C₁-C₄ alkyl and the other is C₁-C₄ alkyl, CF₃ or C₁-C₄ alkoxy; or R and R₁ are the same and are C₁-C₄ alkoxy.

Pharmaceutically acceptable salts of the compounds of formula (I) are their salts with pharmaceutically acceptable, either inorganic or organic, acids.

Examples of inorganic acids are hydrochloric, hydrobromic, sulfuric and nitric acid; examples of organic acids are acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic and p-toluenesulfonic acid.

A particularly preferred n value is 3.

X is preferably chloro or bromo, in particular chloro.

A preferred class of compounds according to the present invention are the compounds of formula (I) wherein:

n is 3;

X is chloro;

one of R and R₁ is hydrogen or C₁-C₄ alkyl and the other is C₁-C₄ alkyl, CF₃ or C₁-C₄ alkoxy; and the pharmaceutically acceptable salt thereof.

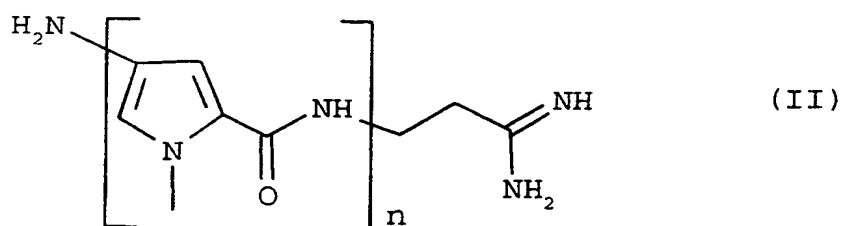
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Examples of specific compounds under this invention, especially in the form of salts preferably with hydrochloric acid, are the following:

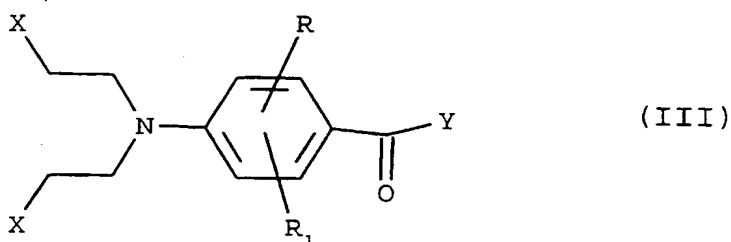
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]
propionamidine; and

β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-
methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]
5 pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine.

The compounds of the invention and the salts thereof can be
obtained by a process comprising reacting a compound of
10 formula (II)



wherein n is as defined above, with a compound of formula
(III)



15 wherein

R , R_1 and X are as defined above and Y is hydroxy or leaving
group; and, if desired, salifying a compound of formula (I)
or obtaining a free compound from a salt thereof, and/or, if
desired, separating a mixture of isomers of a compound of
20 formula (I) into the single isomers.

The reaction of a compound of formula (II) with a compound of
formula (III) can be carried out according to known methods,

by known standard methods.

Well known procedures such as, e.g. fractional crystallization or chromatography may also be followed for separating a mixture of isomers of formula (I) into the
5 single isomers.

The new compounds of formula (I) prepared according to the above described procedures may be as well purified by conventional methods such as, e.g., silica gel or alumina column chromatography, and/or by recrystallization from an
10 organic solvent such as, e.g., a lower aliphatic alcohol, e.g. methyl, ethyl or isopropyl alcohol, or dimethylformamide.

PHARMACOLOGY

15 The compounds of the invention can be useful as antineoplastic and antiviral agents. They show, in particular, cytostatic properties towards tumor cells so that they can be useful, e.g., to inhibit the growth of various tumors, such as, for instance, carcinomas, e.g. mammary
20 carcinoma, lung carcinoma, bladder carcinoma, colon carcinoma, ovary and endometrial tumors in mammals, including humans. Other neoplasias in which the compounds of the invention could find application are, for instance, sarcomas, e.g. soft tissue and bone sarcomas, and the hematological
25 malignancies such as, e.g. leukemias.

The antitumor activity was evaluated in vitro by cytotoxicity studies carried out on murine L1210 leukemia cell. Cells were derived from in vivo tumors and established in cell culture. Cells were used until the tenth passage. Cytotoxicity was
30 determined by counting surviving cells after 48 hours treatment.

The compounds of the invention showed higher antitumor activity in these tumor models than closely related compounds known from EP-B-0246868.

For example, the representative compounds β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 29325) and β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 29721) and the prior art compound, according to EP-B-0246868, β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 24517), were tested against disseminated L₁₂₁₀ murine leukemia showing the following activity data.

Table 1

Compound (internal code)	mg/kg	T/C %	Tox
FCE 29325	3.13	191	0/10
FCE 29721	3.13	183	0/10
FCE 24517	3.13	133	0/10

The activity data occurring in above Table 1 show that the compounds of the instant invention, bearing the claimed substituents on the phenyl ring of the benzoyl mustard moiety, are more active than the closely related unsubstituted prior art compound FCE 24517.

The compounds of the invention show also a remarkable effectiveness in interfering with the reproductive activity

As already said, the pharmaceutical compositions of the invention contain a compound of formula (I) as the active substance, in association with one or more pharmaceutically acceptable excipients.

- 5 The pharmaceutical compositions of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For instance, solutions for intravenous injection or infusion may contain as carrier, for example, sterile water or
10 preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl
15 oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

In the forms for topical application, e.g. creams, lotions or pastes for use in dermatological treatment, the active ingredient may be mixed with conventional oleaginous or
20 emulsifying excipients.

The solid oral forms, e.g. tablets and capsules, may contain, together with the active compound, diluents, e.g., lactose, dextrose, saccharose, cellulose, corn starch and potato starch; lubricants, e.g. silica, talc, stearic acid,
25 magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethyl cellulose, polyvinylpyrrolidone; disaggregating agents, e.g. a starch, alginic acid, alginates, sodium starch glycolate;
30 effervescing mixtures; dyestuffs; sweeteners; wetting agents, for instance, lecithin, polysorbates, laurylsulphates; and,

doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluoro-uracil, melphalan, cyclophosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin and mitomycin or a mixtures of two or more thereof.

- 5 The compounds of the invention can therefore be used in a treatment to ameliorate a cancer. They may be administered to a patient suffering from a cancer treatable with an antitumor agent, for example an anthracycline glycoside such as doxorubicin, daunomycin, epirubicin, 4-demethoxy daunorubicin
10 or idarubicin as mentioned above, together with the antitumor agent.

A compound of the invention and an antitumor agent such as an anthracycline glycoside can be administered to improve the condition of a patient having a leukaemia lymphoma, sarcoma,
15 such as myeloblastic leukaemia, neuroblastoma, Wilm's tumor or malignant neoplasm of the bladder, breast, lung or thyroid.

The following examples illustrate but do not limit the
20 invention.

The abbreviations DMF, DMSO and P.M.R. stand for dimethylformamide, dimethylsulfoxide and proton magnetic resonance respectively.

25 **Example 1**

The compound β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride

The mixture was stirred for one hour and then added of a solution of hydrochloric acid 2N until pH=1. The solvent was evaporated in vacuo and the solid residue purified by flash chromatography on silica gel with a mixture of methylene chloride, methanol, yielding 500 mg of the title compound.

FAB-MS: m/z: 711 (45 [M+H]⁺), 258 (75)

P.M.R. (DMSO) δ : 10.19 (s, 1H); 9.97 (s, 1H); 9.91 (s, 1H); 8.7 (bs, 4H); 8.21 (t, J=5.7 Hz, 1H); 7.74 (m, 2H); 7.29 (d, J=1.8 Hz, 1H); 7.28 (d, J=7.5 Hz, 1H); 7.22 (d, J=1.8 Hz, 1H); 7.17 (d, J=1.8 Hz, 1H); 7.08 (d, J=1.8 Hz, 1H); 7.05 (d, J=1.8 Hz, 1H); 6.94 (d, J=1.8 Hz, 1H); 3.85 (s, 3H); 3.83 (s, 3H); 3.80 (s, 3H); 3.3-3.7 (m, 10H); 2.6 (t, J=6.6 Hz, 2H); 2.33 (s, 3H).

By analogous procedure and using the opportune intermediate the following compounds can be obtained:

β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide hydrochloride

FAB-MS: m/z: 725 (90 [M+H]⁺)

U.V. (EtOH 95%) λ 310; ϵ = 42985

P.M.R. (DMSO) δ : 10.22 (s, 1H); 10.01 (s, 1H); 9.94 (s, 1H); 8.99 (s, 2H); 8.64 (s, 2H); 8.21 (t, J=5.7 Hz, 1H); 7.61 (s, 2H); 7.29 (d, J=1.7 Hz, 1H); 7.21 (d, J=1.7 Hz, 1H); 7.18 (d, J=1.7 Hz, 1H); 7.08 (d, J=1.7 Hz, 1H); 7.05 (d, J=1.7 Hz, 1H); 6.91 (d, J=1.7 Hz, 1H); 3.86 (s, 3H); 3.84 (s, 3H); 3.81 (s, 3H); 3.62 (m, 2H); 3.60-3.30 (m, 8H); 2.62 (m, 2H); 2.35 (s, 6H).

methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine.

5 **Example 2**

Tablets each weighting 0.250 g and containing 50 mg of the active substance can be manufactured as follows:

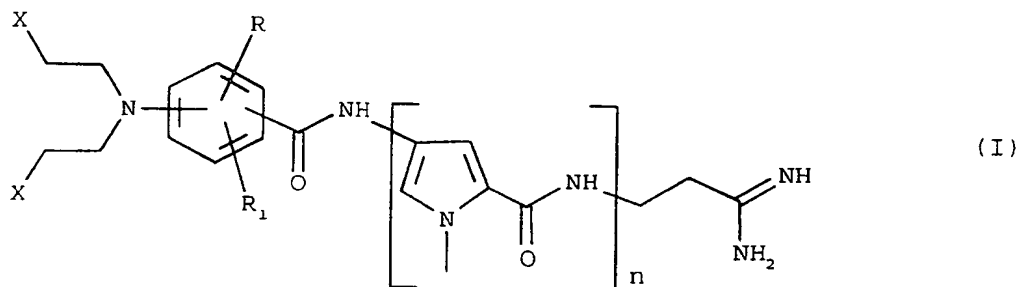
Composition for 10.000 tablets	
β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	500 g
Lactose	1.400 g
Corn starch	500 g
Talc powder	80 g
Magnesium stearate	20 g

- 10 The β -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride, the lactose and half the corn starch are mixed; the mixture is then forced through a sieve
15 of 0.5 mm mesh size.

Corn starch (10 g) is suspended in warm water (90 ml) and the resulting paste is used to granulate the powder. The granulate is dried, comminuted on a sieve of 1.4 mm mesh size, then the remaining quantity of starch, talc and
20 magnesium stearate is added, carefully mixed and processed into tablets.

CLAIMS

1. A compound of formula (I)



5 wherein

n is 2, 3 or 4;

one of R and R₁ is hydrogen, C₁-C₄ alkyl, CF₃ or C₁-C₄ alkoxy and the other is independently CF₃, C₁-C₄ alkyl or C₁-C₄ alkoxy; and

10 X is halogen and the pharmaceutically acceptable salts thereof.

2. A compound of formula (I), according to claim 1, wherein

15 n is 3;

X is chloro;

one of R and R₁ is hydrogen or C₁-C₄ alkyl and the other is C₁-C₄ alkyl, CF₃ or C₁-C₄ alkoxy; and the pharmaceutically acceptable salts thereof.

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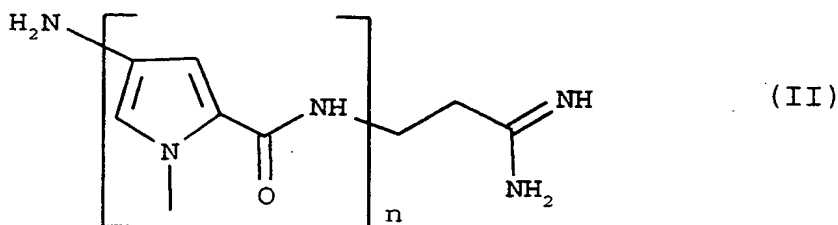
3. A compound selected from:

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]

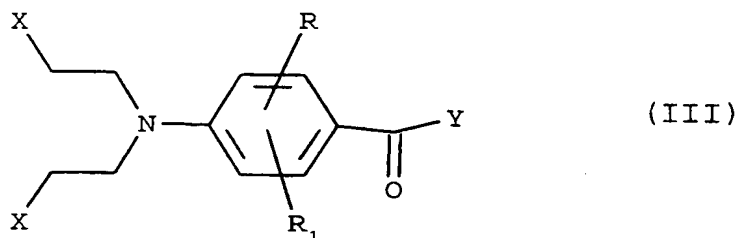
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine; or a pharmaceutically acceptable salt thereof.

4. A salt of a compound according to claim 3, wherein said salt is the hydrochloride.

5. A process for the preparation of a compound of formula (I), according to claim 1, or a salt thereof, said process comprising reacting a compound of formula (II)



wherein n is as defined in claim 1, with a compound of formula (III)



wherein

R, R₁ and X are as defined in claim 1 and Y is hydroxy or leaving group; and, if desired, salifying a compound of formula (I) or obtaining a free compound from a salt thereof, and/or, if desired, separating a mixture of isomers of a compound of formula (I) into the single isomers.

INTERNATIONAL SEARCH REPORT

International Application No
PL 1/EP 96/02659

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07D207/34 A61K31/40		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. MED. CHEM. (1989), 32(4), 774-8 CODEN: JMCMAR;ISSN: 0022-2623, 1989, XP000608784 ARCAMONE, FEDERICO MARIA ET AL: "Synthesis, DNA-binding properties, and antitumor activity of novel distamycin derivatives" see the whole document ---	1-10
A	EP 0 246 868 A (FARMITALIA CARLO ERBA S.P.A., ITALY) 25 November 1987 cited in the application see the whole document -----	1-10
<div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> Further documents are listed in the continuation of box C. </div> <div> <input checked="" type="checkbox"/> Patent family members are listed in annex. </div> </div>		
<div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>* & * document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">7 November 1996</div>	Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">19.11.1996</div>	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer <div style="text-align: center; font-weight: bold;">Kissler, B</div>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PL 1/EP 96/02659

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0246868	25-11-87	AU-B- 597659	07-06-90
		AU-A- 7316387	26-11-87
		BG-B- 60531	28-07-95
		CA-A- 1314551	16-03-93
		DE-A- 3781716	22-10-92
		HK-A- 31993	08-04-93
		IE-B- 60198	15-06-94
		JP-B- 6023193	30-03-94
		JP-A- 62294653	22-12-87
		KR-B- 9511408	04-10-95
		SU-A- 1528316	07-12-89
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